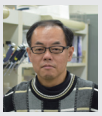
## LABORATORY OF ORGANELLE REGULATION



*Associate Professor*
MANO, Shoji

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|---|---|
| *NIBB Research Fellow:* | *KANAI, Masatake* |
| *Postdoctoral Fellow:* | *KANAI, Masatake** |
| *Visiting Scientist:* | *KAMIGAKI, Akane* |
| *Technical Assistant:* | *HIKINO, Kazumi* |
| | *NAGATA, Kyoko* |
| *Secretary:* | *UEDA, Chizuru* |
| | *ASAI, Sanae* |

Because plants spread their roots in the ground, they must survive in a given environment. To adapt to their environment, they recognize environmental changes as important signals that are necessary for their survival. In such cases, plant cells can induce, degenerate and differentiate their organelles. The flexibility of plant organelles is the basis of the strategy for environmental adaptation in plants.

The aims of this laboratory are to clarify the molecular mechanisms underlying the induction, differentiation, and interaction of organelles, especially peroxisomes and oil bodies, and to understand the integrated functions of individual plants through organelle dynamics.

### I. Molecular mechanisms of peroxisome dynamics and functions in plant cells

Peroxisomes are single-membrane bounded organelles, which are ubiquitously present in eukaryotic cells, and are involved in various biological processes such as lipid metabolism and photorespiration. These functions are dramatically changed in developmental stages and when confronted with environmental changes. For example, light induces transformation of peroxisomes from glyoxysomes, which are peroxisomes engaged in the degradation of reserve oil stored in the oil body via β-oxidation and the glyoxylate cycle, to another type of peroxisome, leaf peroxisomes, that function in several crucial steps of photorespiration. After the functional transformation of glyoxysomes to leaf peroxisomes during the greening of cotyledons, the reverse transformation of leaf peroxisomes to glyoxysomes occurs during senescence. Gene expression, alternative splicing, protein translocation, protein degradation and degradation of peroxisomes themselves control these functional transformations.

To better understand peroxisome biogenesis and functions, we isolated a number of Arabidopsis mutants that displayed aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual positioning (*peup* mutants) based on them having a different pattern of GFP fluorescence from the parent plant, GFP-PTS1, in which peroxisomes with normal sizes, numbers and distribution could be visualized with GFP.

As of writing, we have reported the function of APEM1, APEM2, APEM3, APEM4, APEM9 and APEM10 (Figure 1). Based on the results we were able to update the model for functional transformation of peroxisomes using these *apem* mutants in concert with the analyses of *peup1*, *peup2* and *peup4* mutants, which were defective in Autophagy-related 2 (ATG2), ATG18a and ATG7, respectively.

We are currently investigating other *apem* and *peup* mutants. From these analyses, we will be able to identify the components responsible for peroxisome biogenesis, functions and maintenance, and to address the mechanism at the molecular level.

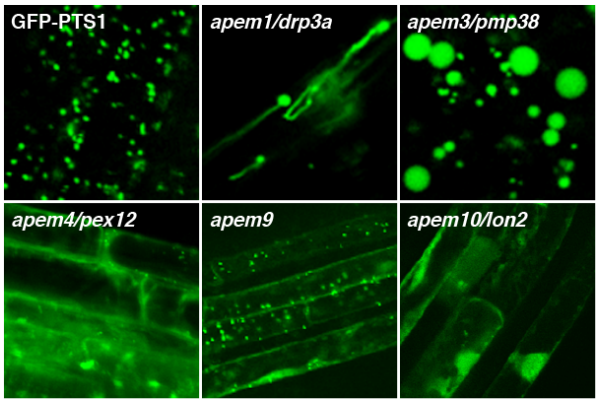


Figure 1. Phenotype of Arabidopsis *apem* mutants. GFP fluorescence was observed in the parent plant, GFP-PTS1, *apem1*, *apem3*, *apem4*, *apem9* and *apem10* mutants. *apem1* and *apem3* have elongated and enlarged peroxisomes, respectively. In *apem4*, *apem9* and *apem10*, GFP fluorescence is observed in the cytosol because of the decrease of the efficiency of protein transport to peroxisomes.

### II. Accumulation mechanism of seed storage oils and proteins

Plant seeds accumulate huge amounts of storage reserves such as oils, carbohydrates and proteins. Humans use these storage reserves in food and industrial materials. Storage reserves vary among different types of plant seeds. Wheat, maize and rice seeds mainly accumulate starch, whereas rapeseed, pumpkin and sesame contain large amounts of oils. Soybeans contain proteins as a major reserve. Storage oils and storage proteins are synthesized in the endoplasmic reticulum (ER) and accumulated in oil bodies and protein bodies, respectively, during the same period of seed development (Figure 2).

We are analyzing the molecular mechanisms controlling oil and protein contents in seeds. Based on the analysis of the temporal sequence of oil and protein synthesis during seed development in *Arabidopsis thaliana*, which produces seeds containing approximately 30% oil and 30% protein, we

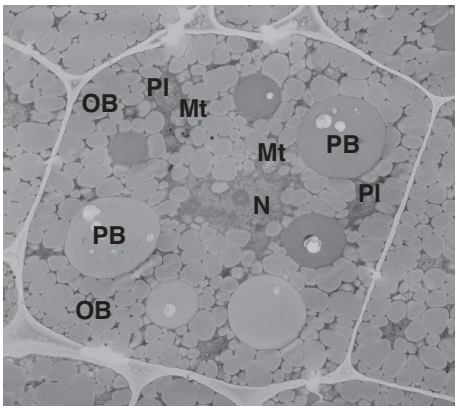


Figure 2. Electron micrograph of Arabidopsis dry seed. Mt, mitochondrion; N, nucleus; OB, oil body; PB, protein body; Pl, plastid.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

revealed that the extension of *WRINKLED1* (*WRI1*), a transcription factor in fatty acid biosynthesis, expression during the midphase of seed development significantly enhanced seed oil content, and caused an enlargement of seed size.

We are also currently investigating the mechanisms of oil accumulation in other plant species. In addition, we are trying to apply our knowledge and techniques to increase beneficial storage reserves.

## III. Development of Gateway-technology vectors for plant research

Gateway cloning is a popular technology, which allows the simultaneous generation of multiple constructs containing a range of fusion genes. We have developed various types of Gateway cloning-compatible vectors for the improvement of resources in the plant research field. As of writing, we have provided vector sets to detect multiple protein-protein interactions in vivo using multi-color bimolecular fluorescence complementation, and the binary vectors to facilitate tripartite DNA assembly and promoter analysis with various reporters and tags in the liverwort *Marchantia polymorpha* (Figure 3). We will continue developing other useful Gateway cloning-compatible vectors to contribute to the plant research community.

## IV. Construction of The Plant Organelles Database 3 (PODB3) and Plant Organelles World

The Plant Organelles Database 3 (PODB3) was built to promote a comprehensive understanding of organelle dynamics. This public database is open to all researchers. PODB3 consists of six individual units: the electron micrograph database, the perceptive organelles database, the organelles movie database, the organellome database, the functional analysis database, and external links. The function of each database is as follows:

- The electron micrograph database provides information on the ultrastructures in plant cells.
- The perceptive organelles database shows organelles dynamics responding to environmental stimuli.
- The organelles movie database contains time-lapse images and 3D structure rotations.
- The organellome database is a compilation of static image data of various tissues of several plant species at different developmental stages.
- The functional analysis database is a collection of protocols for plant organelle research.

Through these databases, users can easily grasp plant organelle dynamics. Plant Organelles World, which is built based on PODB3, is an educational tool for engaging members of the non-scientific community to explore plant biology. We hope that both PODB3 and Plant Organelles World are of help to researchers as well as the general public.

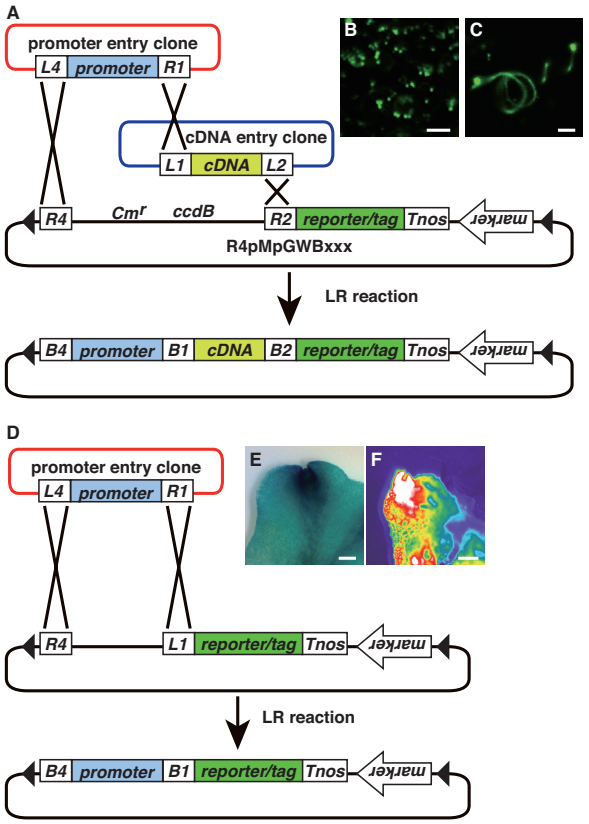


Figure 3. R4pMpGWB and R4L1pMpGWB systems for construction of fusion genes in *M. polymorpha*. (A) In the R4pMpGWB system, promoter and cDNA entry clones and R4pMpGWB vectors are used in a tripartite LR reaction to form a C-terminal fusion of cDNA-encoded protein and a reporter or tag. (B) Thallus epidermal cells expressing the peroxisome-targeted Citrine under the 35S promoter. (C) Sperm cell expressing Lifeact-Venus under the endogenous *PROTAMINE* promoter Bars: 10 μm for (B) and 2 μm for (C). (D) In the R4L1pMpGWB system, the promoter entry clones and R4L1pMpGWB vectors are used for a bipartite LR reaction. (E) GUS staining in thalli expressing *pro*Mp*EF1α:GUS*. (F) Luminescence images after heat shock of transgenic plants bearing *pro*Mp*HSP17.8A1:ELuc(PEST)*. Bars: 1 mm

## Publication List:

### 〔Original paper〕


- Fujikawa, Y., Suekawa, M., Endo, S., Fukami, Y., Mano, S., Nishimura, M., and Esaka, M (2018). Effect of mutation of C-terminal and heme binding region of Arabidopsis catalase on the import to peroxisomes. Biosci. Biotechnol. Biochem. *83*. 322-325.
- Mano, S., Nishihama, R., Ishida, S., Hikino, K., Konodo, M., Nishimura, M., Yamato, T.K., Kohchi, K., and Nakagawa, T. (2018). Novel gateway binary vectors for rapid tripartite DNA assembly and promoter analysis with various reporters and tags in the liverwort *Marchantia polymorpha*. PLoS ONE, *13*, e0204964.


### 〔Review article〕


- Goto-Yamada, S., Hikino, K., Nishimura, M., Nakagawa, T., and Mano, S. (2018). Bimolecular fluorescence complementation with improved gateway-compatible vectors to visualize protein–protein interactions in plant cells. In Methods in Molecular Biology. Two-Hybrid Systems. Methods and Protocol. Oñate-Sánchez, L., ed. (U.S.A., Springer), pp. 245-258.